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Evaluation of Levofloxacin Pharmacodynamics in a mouse Model of Inhalational *Bacillus anthracis*

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Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01 OCT 2005	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Evaluation of Levofloxacin Pharmacodynamics in a mouse Model of Inhalational Bacillus anthracis	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **US Army Medical Research Institute of Infectious Diseases**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES
See also ADM001851, Proceedings of the 2003 Joint Service Scientific Conference on Chemical & Biological Defense Research, 17-20 November 2003. , The original document contains color images.

14. ABSTRACT

15. SUBJECT TERMS

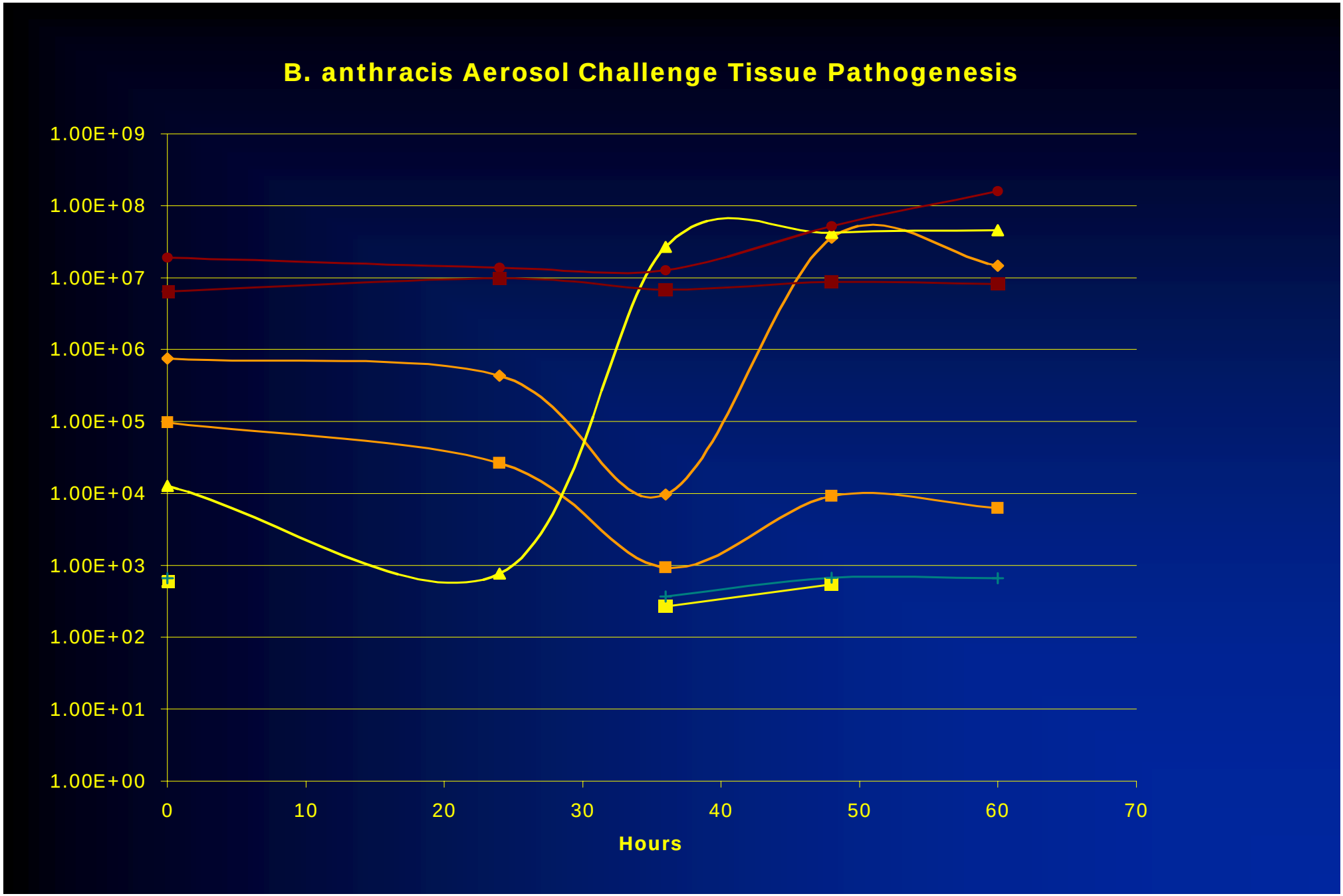
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**UU**	**23**	

Antibiotic Assessment Plan

- Antibiotics licensed or in clinical human trials in the U.S.
- Screen antibiotics by in-vitro assay (MICs)
- Best candidates by MIC and pharmacokinetics (PK) tested in mouse aerosol challenge model
- Best candidates in mouse model tested in non-human Primate model.

Balb/c

- Inbred
- Intermediate sensitivity LD_{50} $8x10^4$
- Consistent with previous data by subcutaneous challenge (Welkos SL; I&I 1986)


B. anthracis Aerosol Challenge Tissue Pathogenesis
1.00E+09
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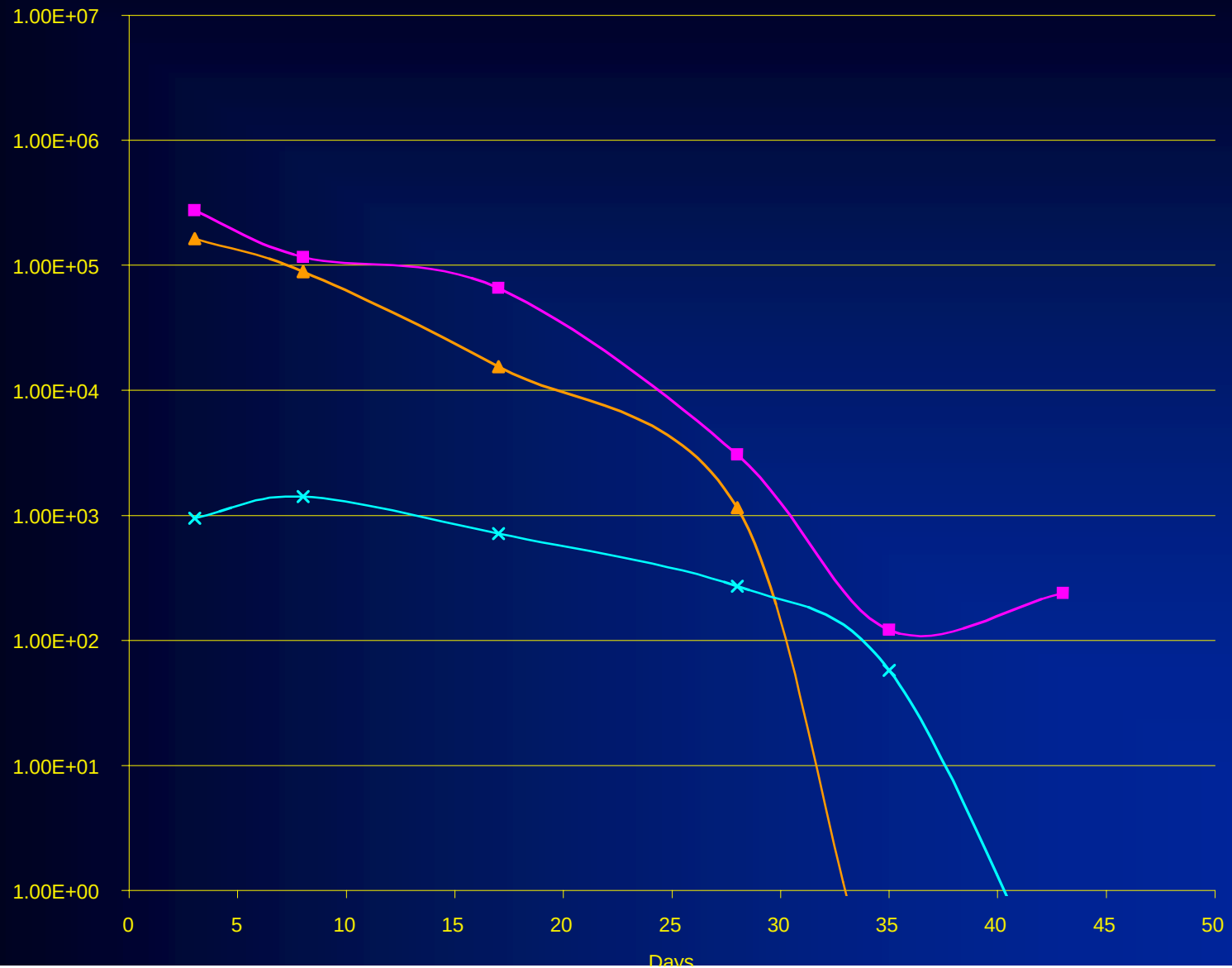
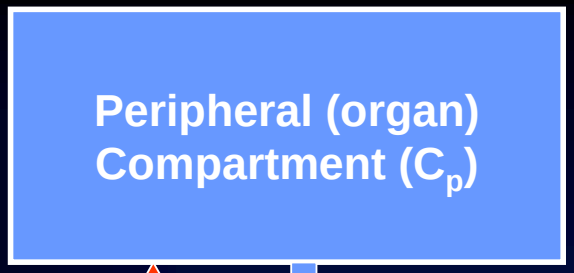
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
0
10
20
30
40
50
60
70
Hours

Treated Infection Burden



Conclusions

- **Balb/c mouse is a suitable model**
  - For inhalational anthrax
  - For screening of antibiotic efficacy in a rodent model
  - As a first step before evaluation of antibiotic efficacy in non-human primates
- **Ciprofloxacin 30mg/Kg, Q12 is an appropriate standard of effective treatment**
  - 21 days sufficient
  - 14 days not sufficient
- **Anthrax spore burden in the lung/mediastinum does not appear to be significantly affected by antibiotic treatment**



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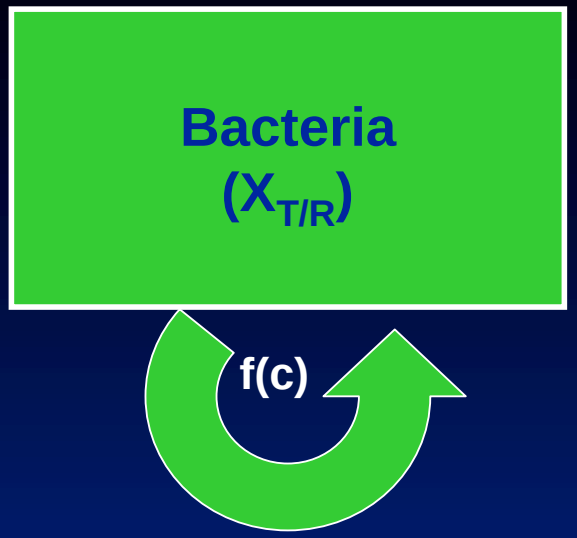


k_{cp} k_{pc}

IP injection

k_e

[1] $\frac{dC_a}{dt} = -k_a C_a$

[2] $\frac{dC_c}{dt} = k_a C_a + k_{pc} Cp - k_{cp} C_c - k_e C_c$

[3] $\frac{dC_p}{dt} = k_{cp} C_c - k_{pc} C_p$

$\frac{dX_S}{dt} = K_{GS} \times X_S \times L - f_{KS}(C_c^{H\xi}) \times X_S$ [4]

$\frac{dX_R}{dt} = K_{GR} \times X_R \times L - f_{KR}(C_c^{H\xi}) \times X_R$ [5]

$L = (1 - (X_R + X_S)/POPMAX)$ [6]

$f_{\psi\xi}(C_c^{H\xi}) = \frac{K_{max\,\xi} \bullet C_c^{H\xi}}{C^{H\xi}_{50\xi} + C_c^{H\xi}}$, ψ=K and ξ = S,R [7]

$Y_1 = X_T = X_S + X_R$ [8]

$Y_2 = X_R$ [9]

Terminology

MIC-Minimum Inhibitory Concentration

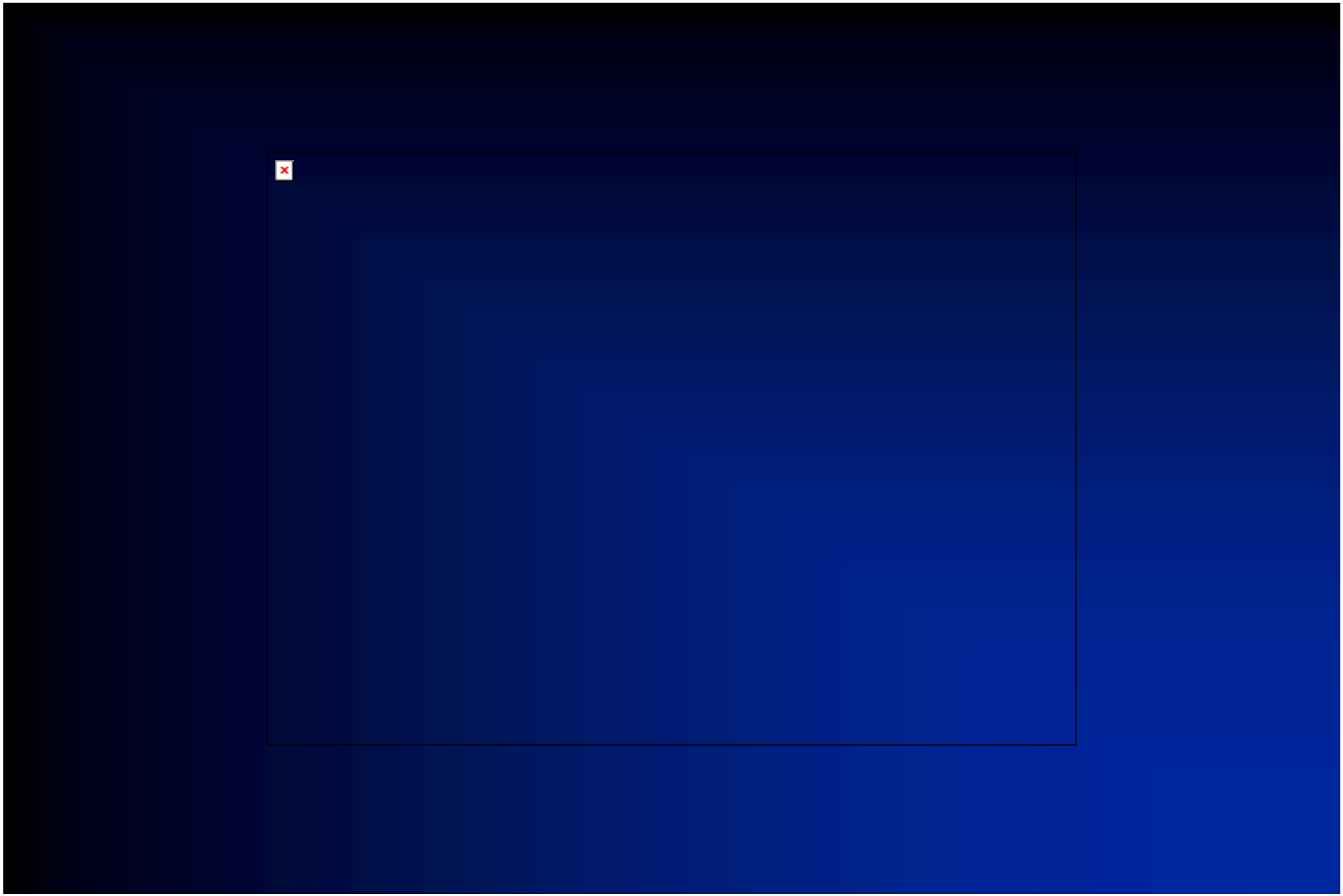
Cmax-Maximum concentration (PK)

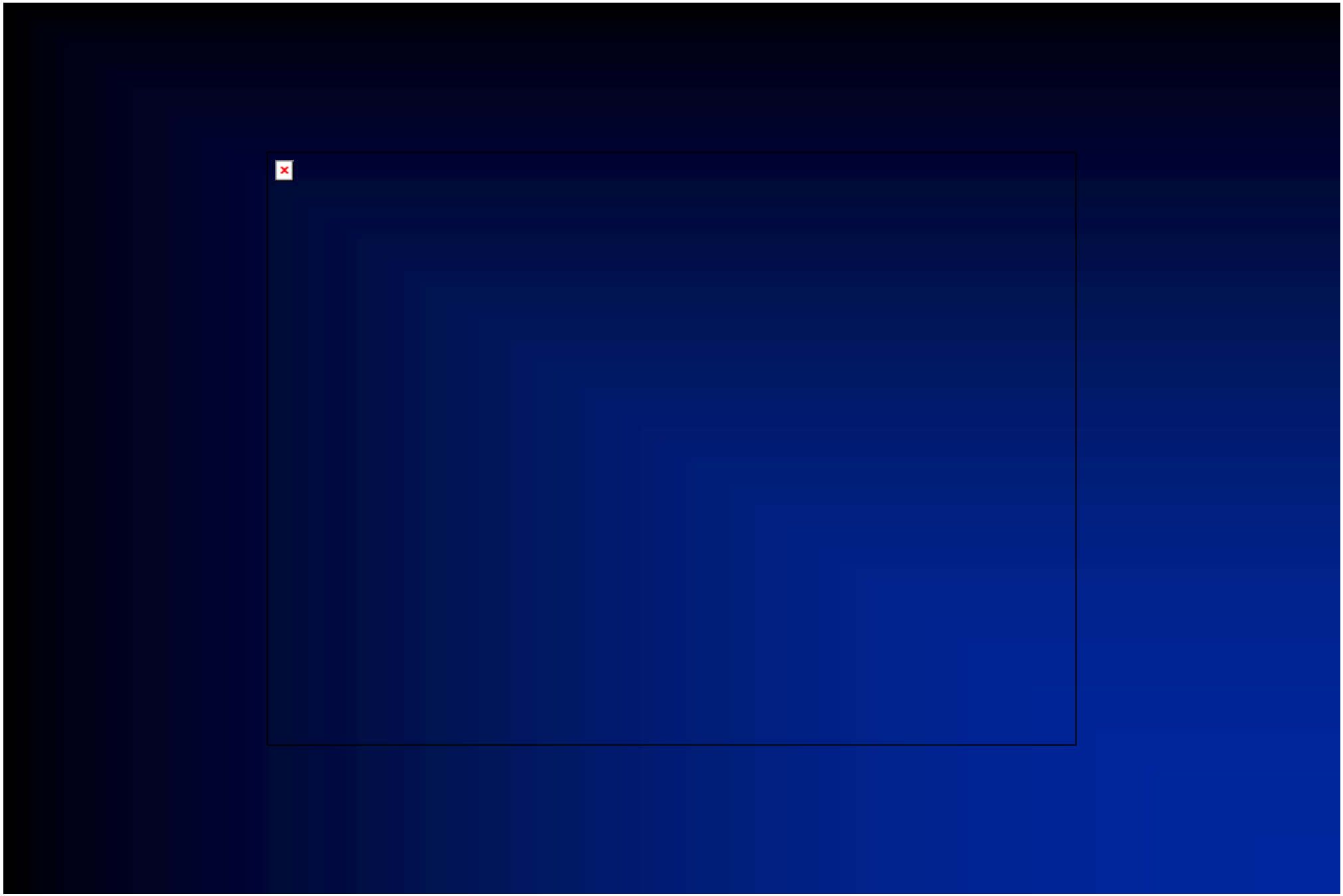
AUC- Area under the Curve (PK)

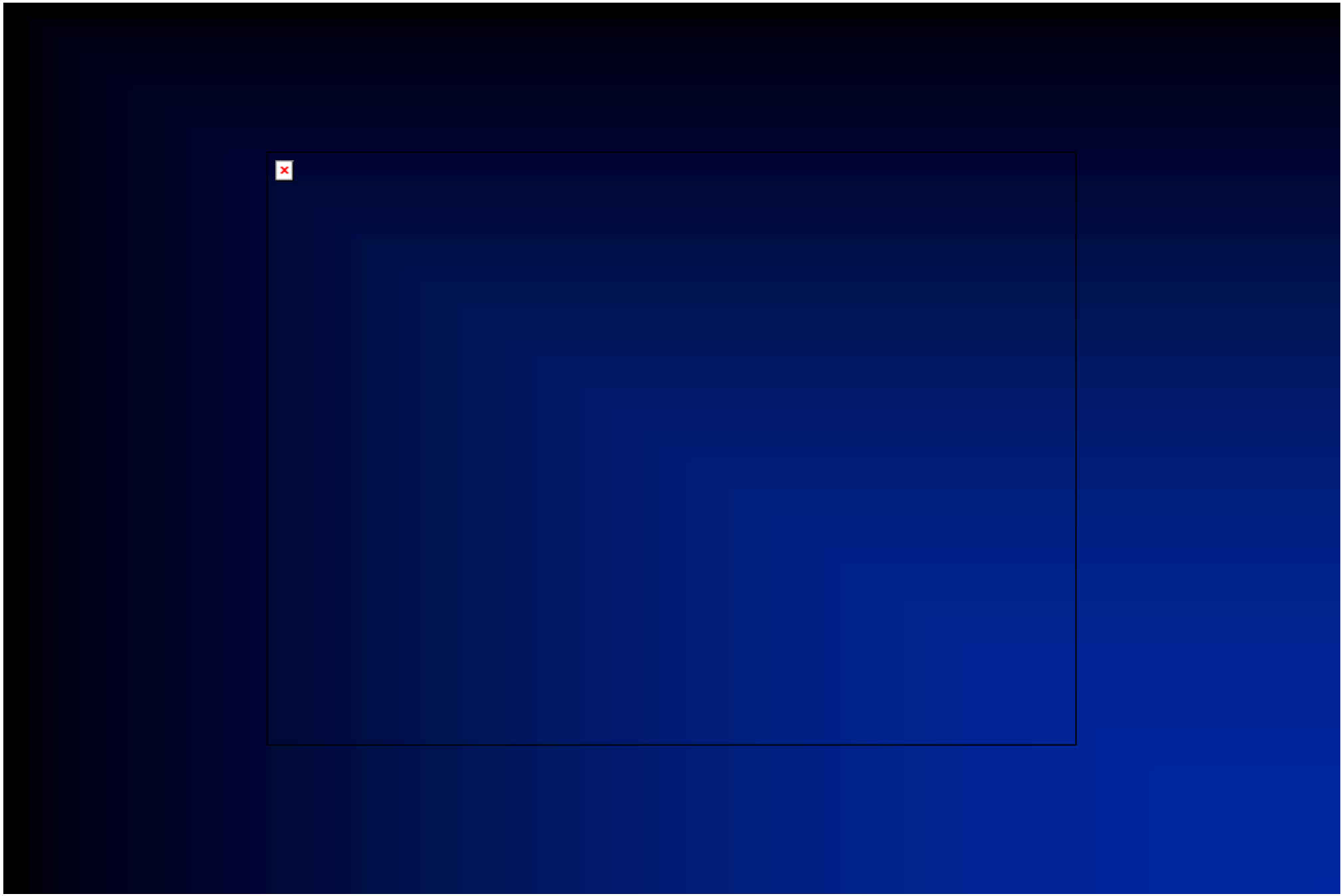
ΔTMIC-Time above MIC (PK)

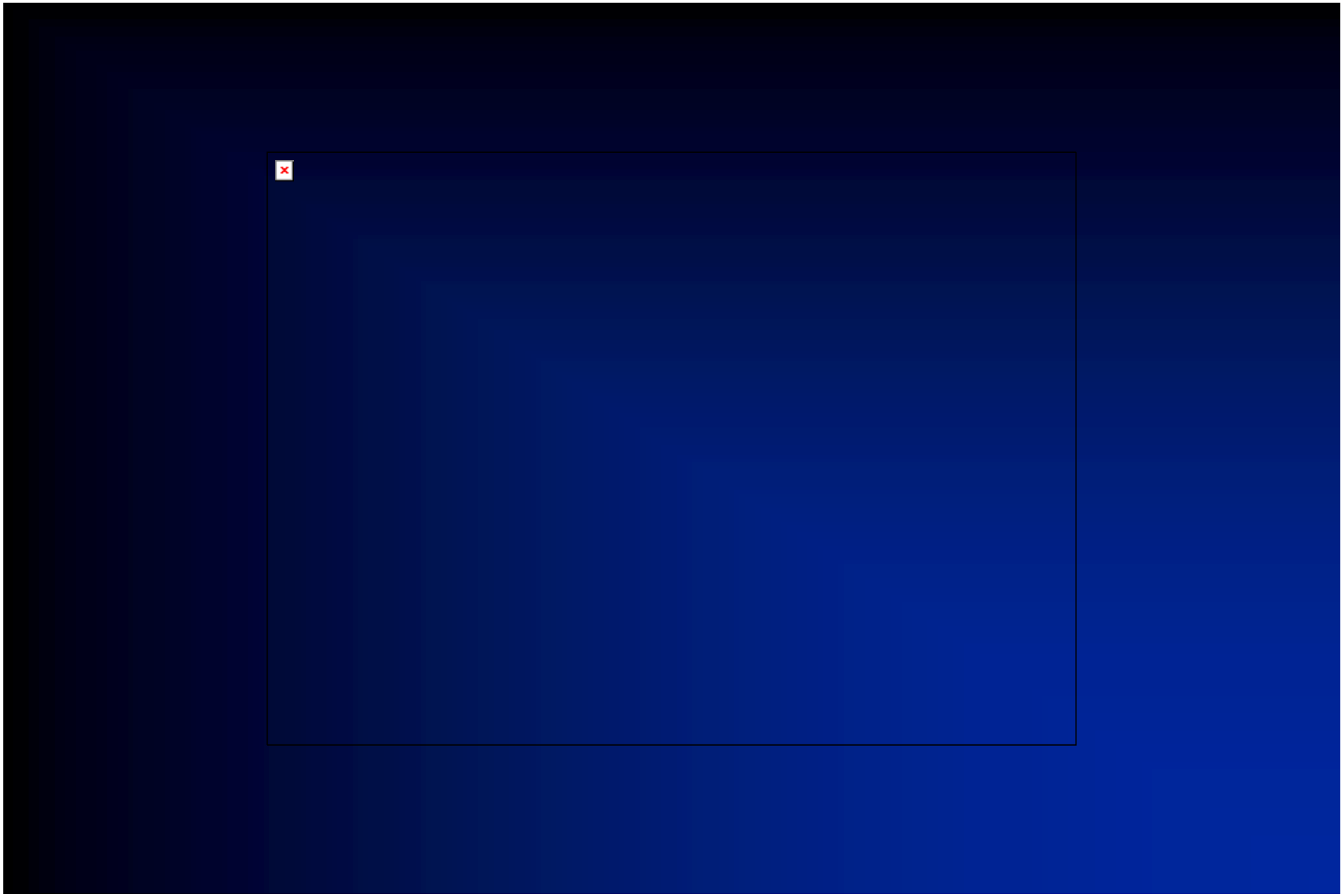
AUC/MIC ratio

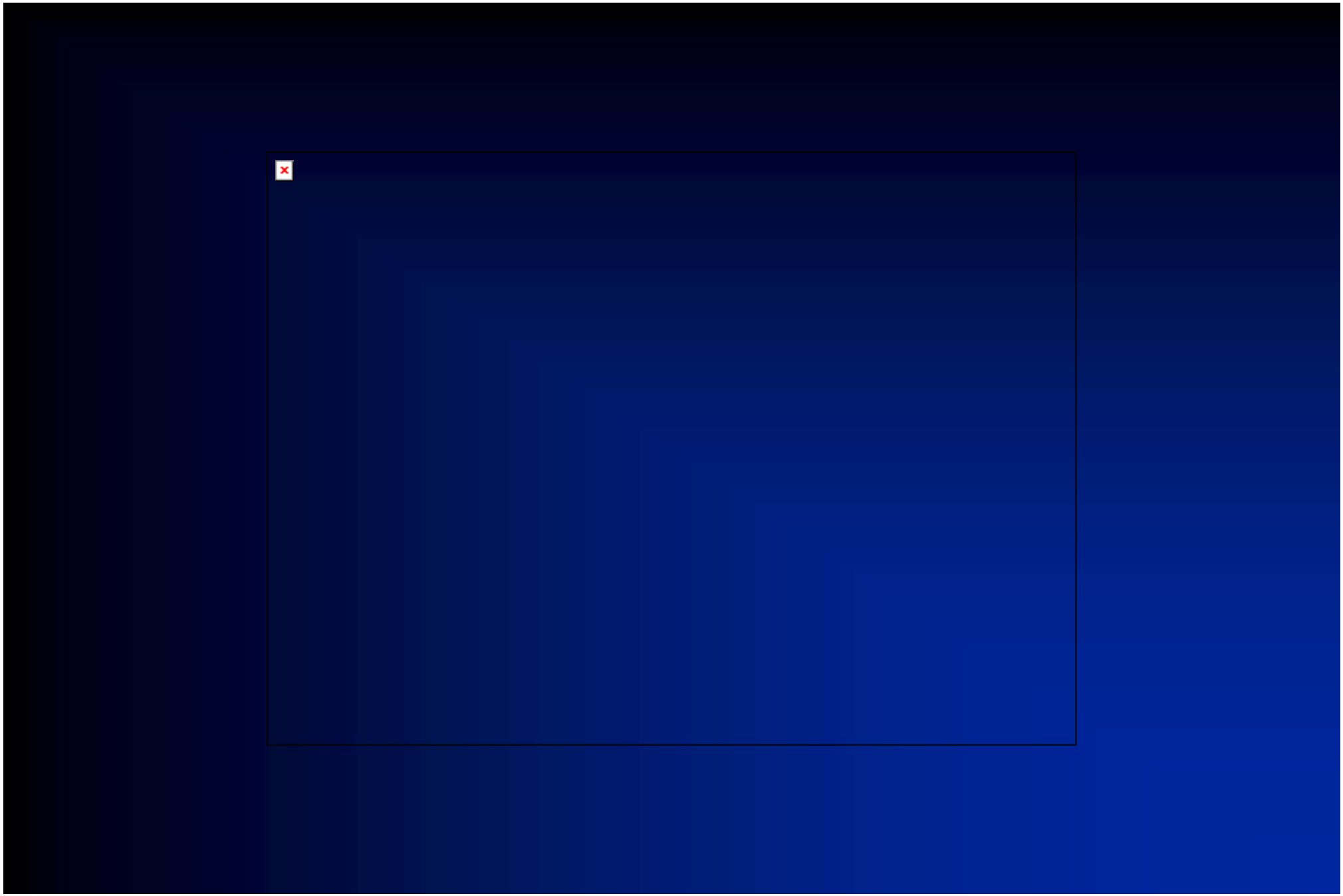
Cmax/MIC

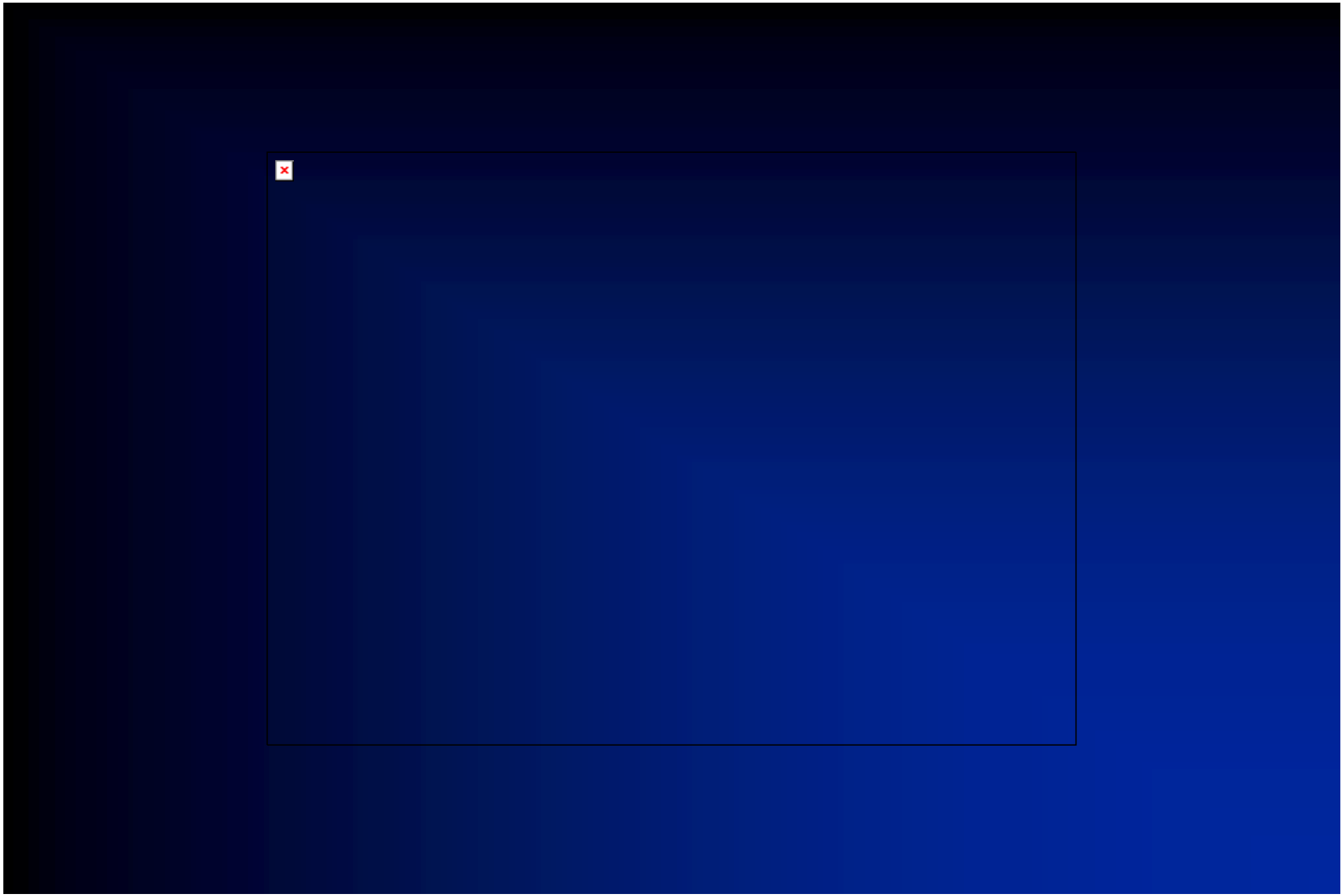


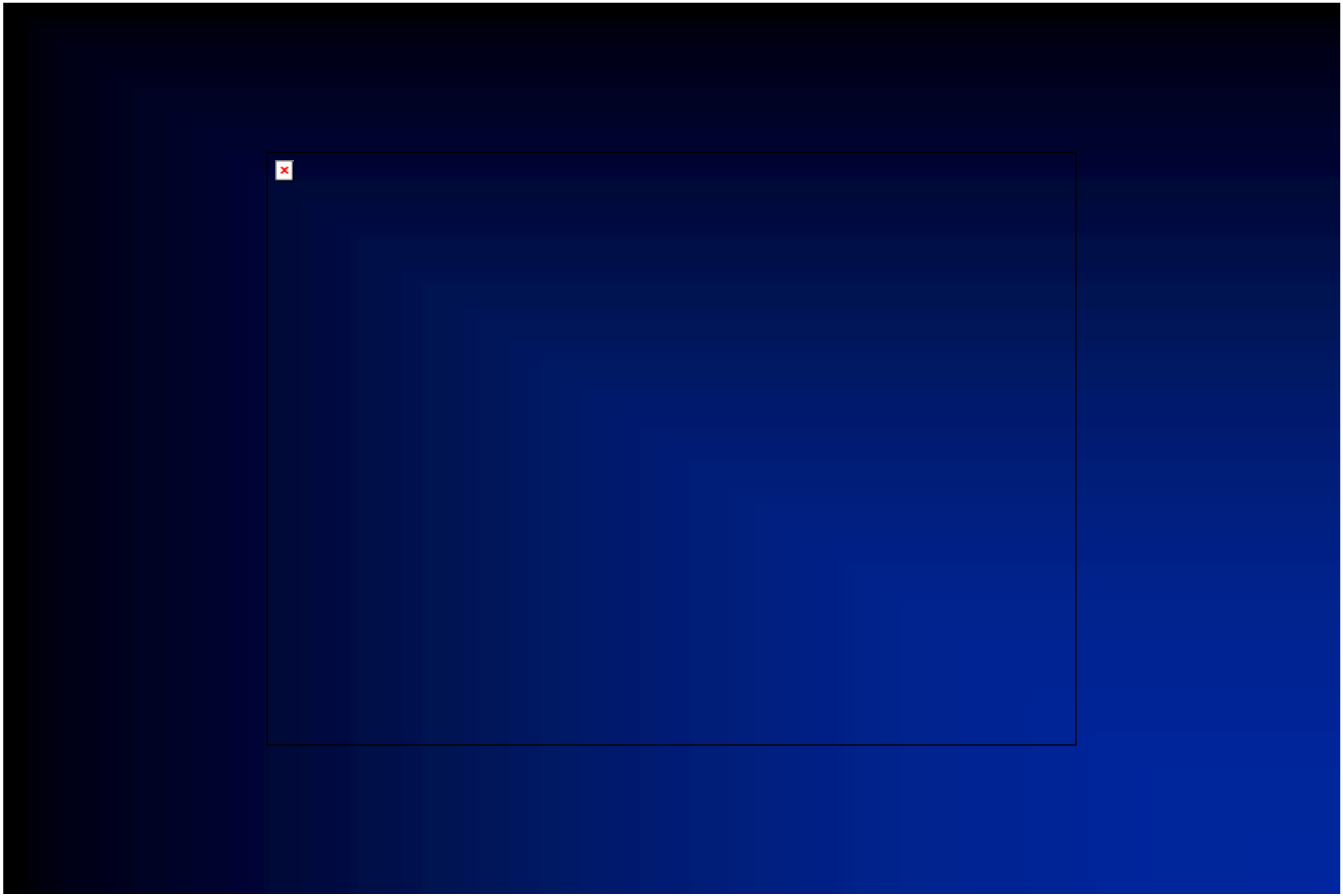






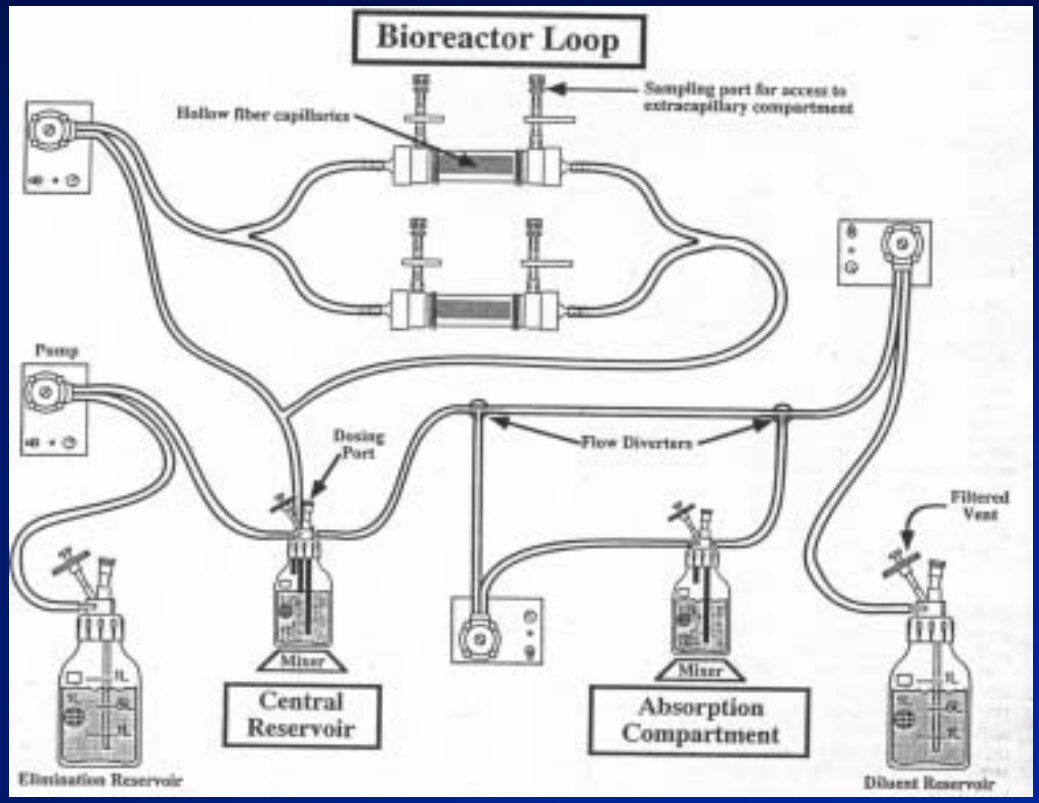






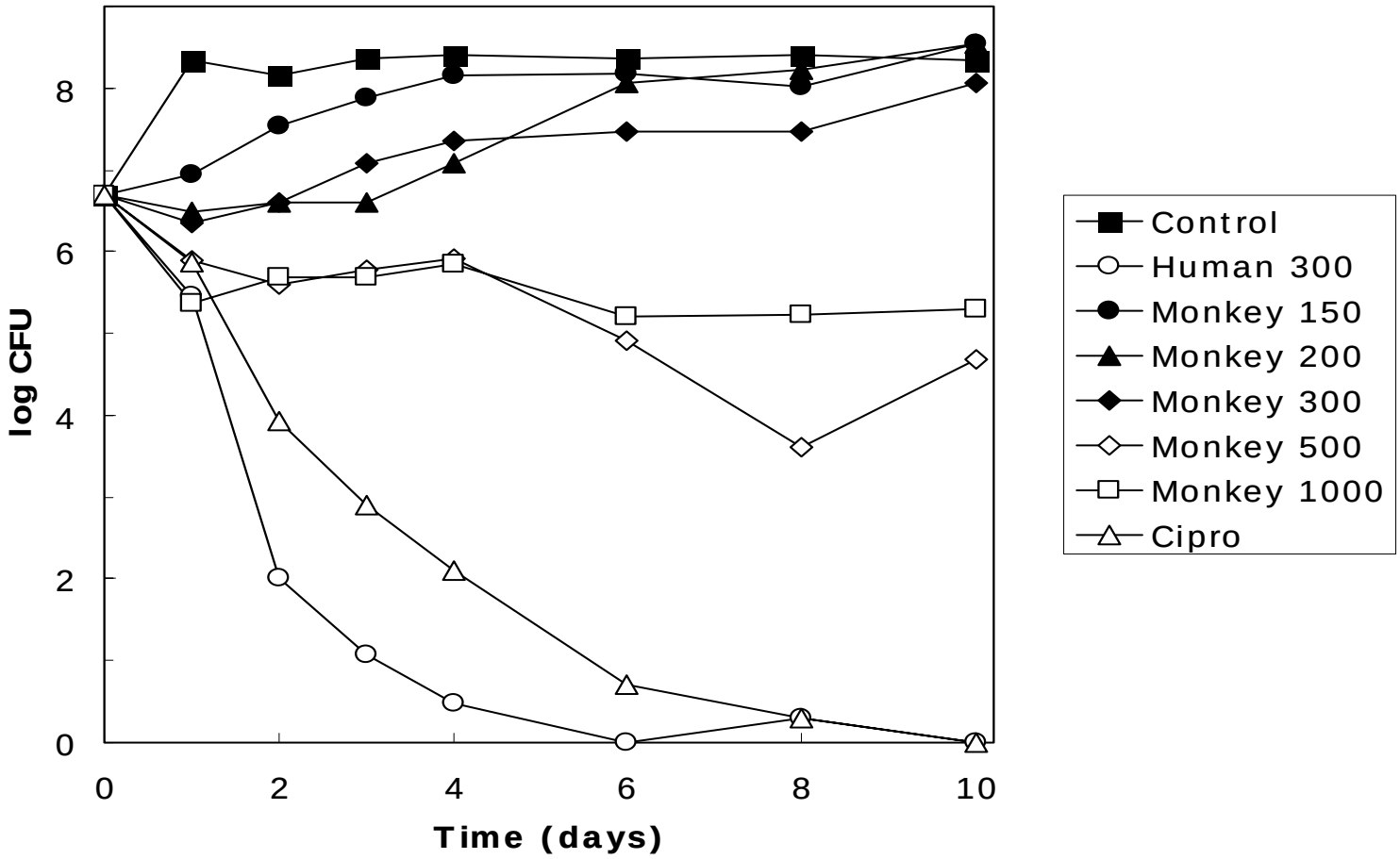
Hollow fiber System allows simulation of human PK *in vitro*

- **Useful for dose ranging and schedule dependency determinations**
- **Allows examination of different classes (beta lactams, fluoroquinolones, etc.)**

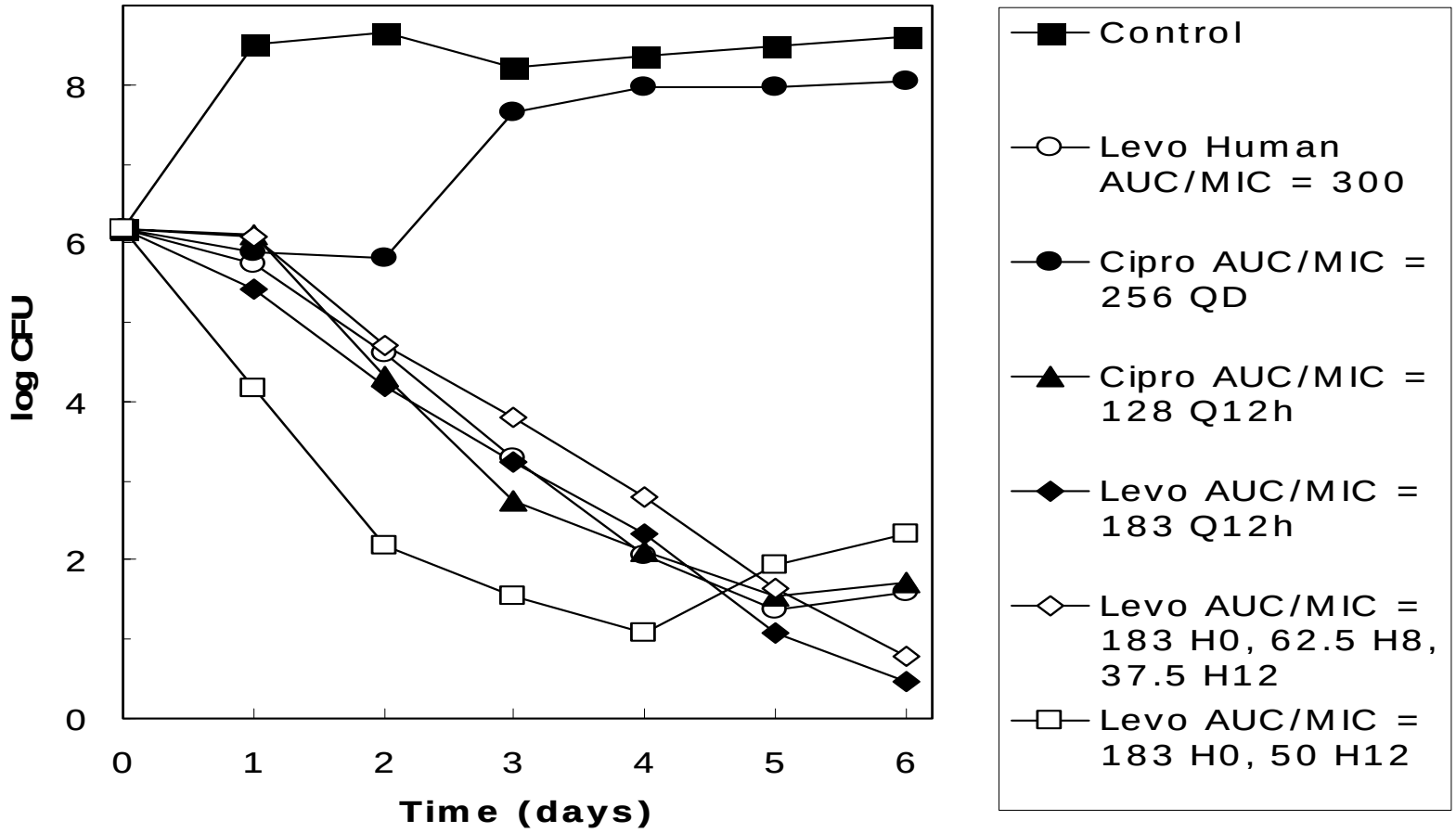


The original hollow fiber system was used by Blaser, Dudley & Zinner

Levo vs. B. anthracis



Levo vs. B. anthracis

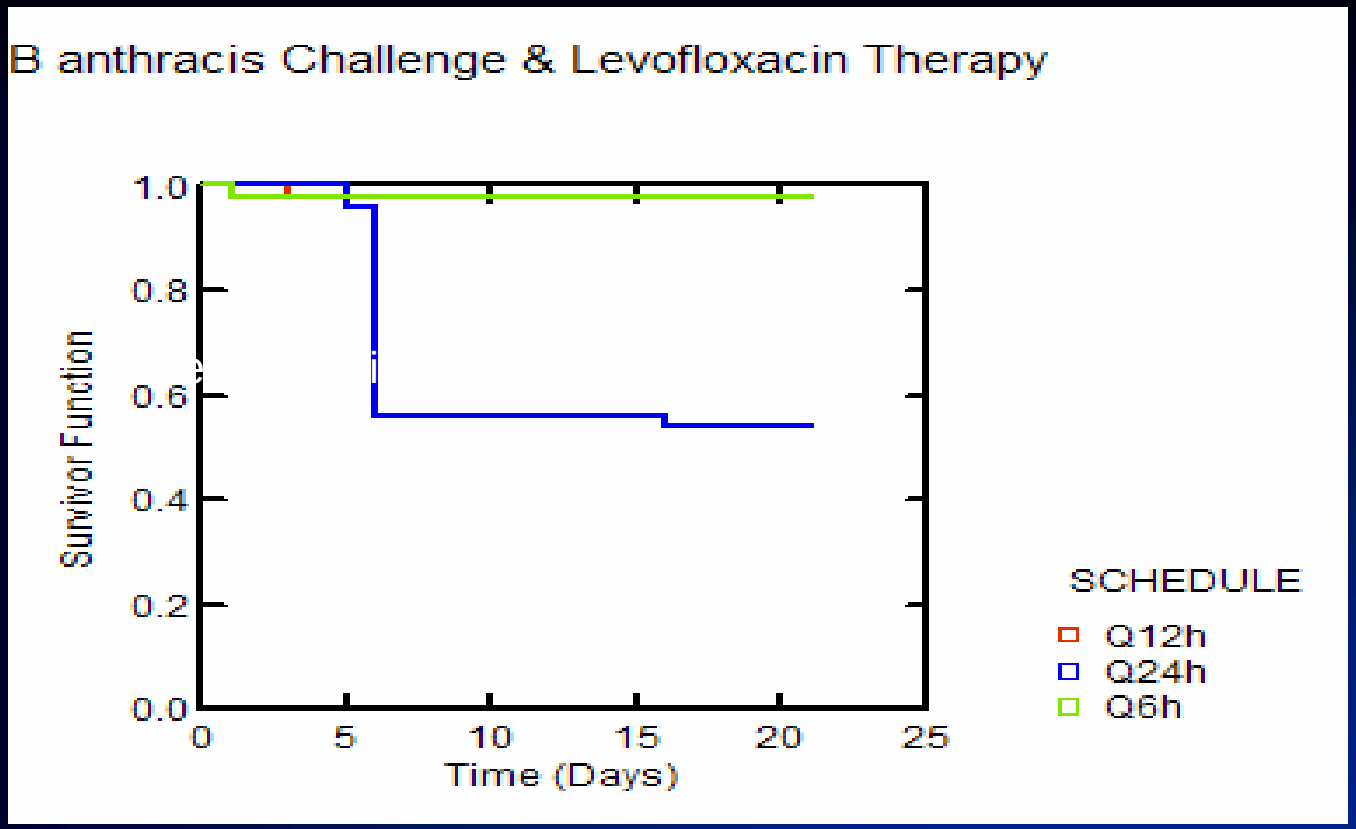


Preliminary Conclusions

- Levofloxacin achieved stasis (at best) on a once-daily schedule of administration with rhesus pharmacokinetics
- Levofloxacin eradicated *Bacillus anthracis* when human PK was employed
- Emergence of resistance was seen for the lower AUC/MIC ratio regimens in a time-dependent manner for levofloxacin

- **In order to determine the validity of these findings, we designed and carried out an aerosol challenge of the Ames strain of *Bacillus anthracis* in a Balb/c mouse model.**
- **The challenge amount was 50-75 LD_{50} of Ames spores (LD_{50} = 8 x 10^4 spores)**
- **Schedules of Q6h, Q12h and Q24h were evaluated,**
- **Total daily doses of 37.5, 75, 150, 225, 300 mg/Kg**

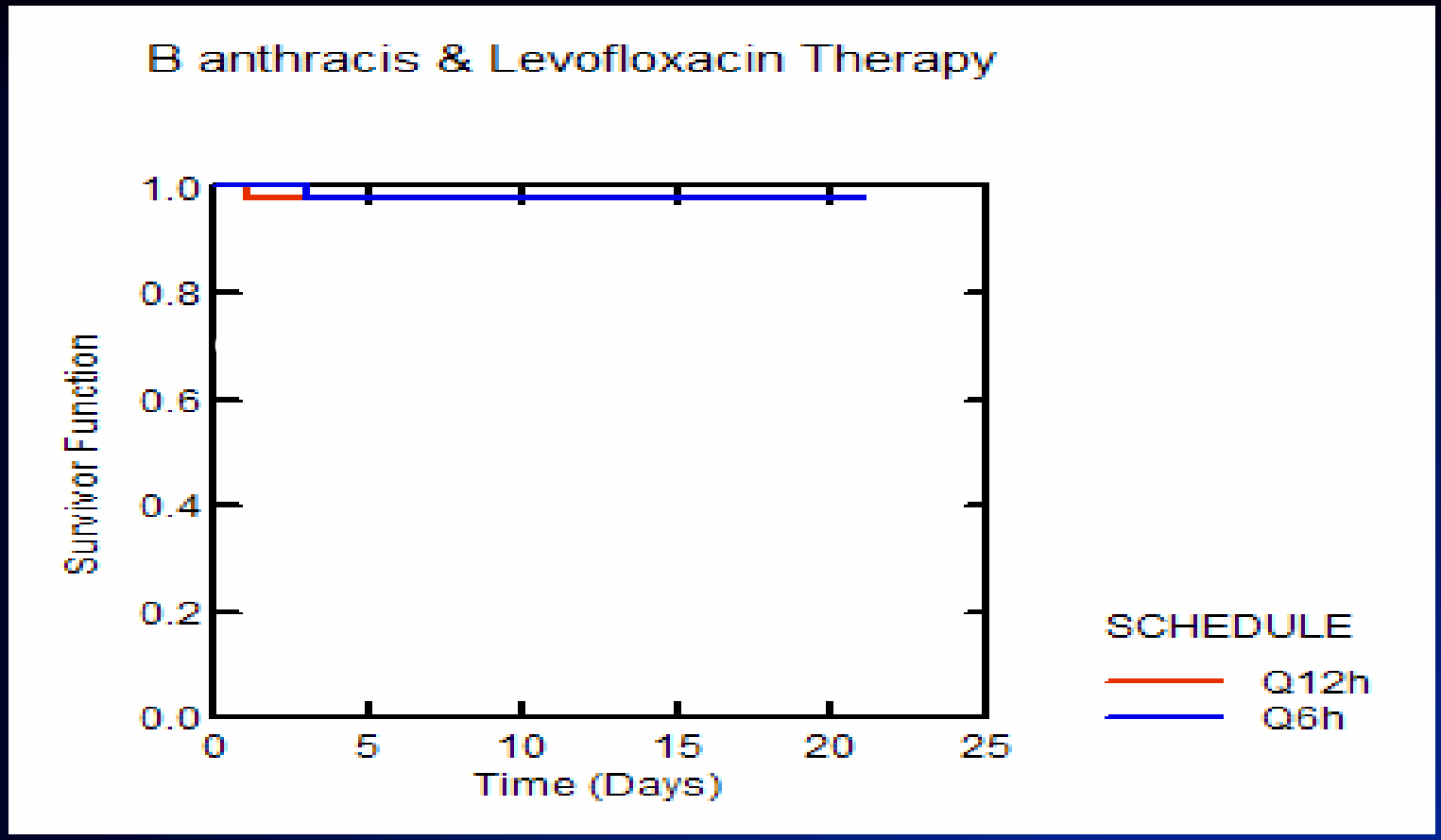
- Population modeling of mouse levofloxacin PK was performed (T1/2 = 1.1[mean PK values]-1.8 [median PK values] h)
- AUC/MIC ratios of 0 (control) to 176 were examined on each administration schedule
- Treatment was for 21 days
- Surviving animals were followed off therapy
- Time to death was one endpoint examined by stratified Kaplan-Meier analysis and also by Cox Proportional Hazards Modeling



Schedule of administration was highly significant ($p < 0.000001$)

Schedule is best handled as a stratification variable, not as a covariate

AUC/MIC was not significant alone, but was a a covariate when added to Schedule as a stratification variable ($p = 0.0012$)

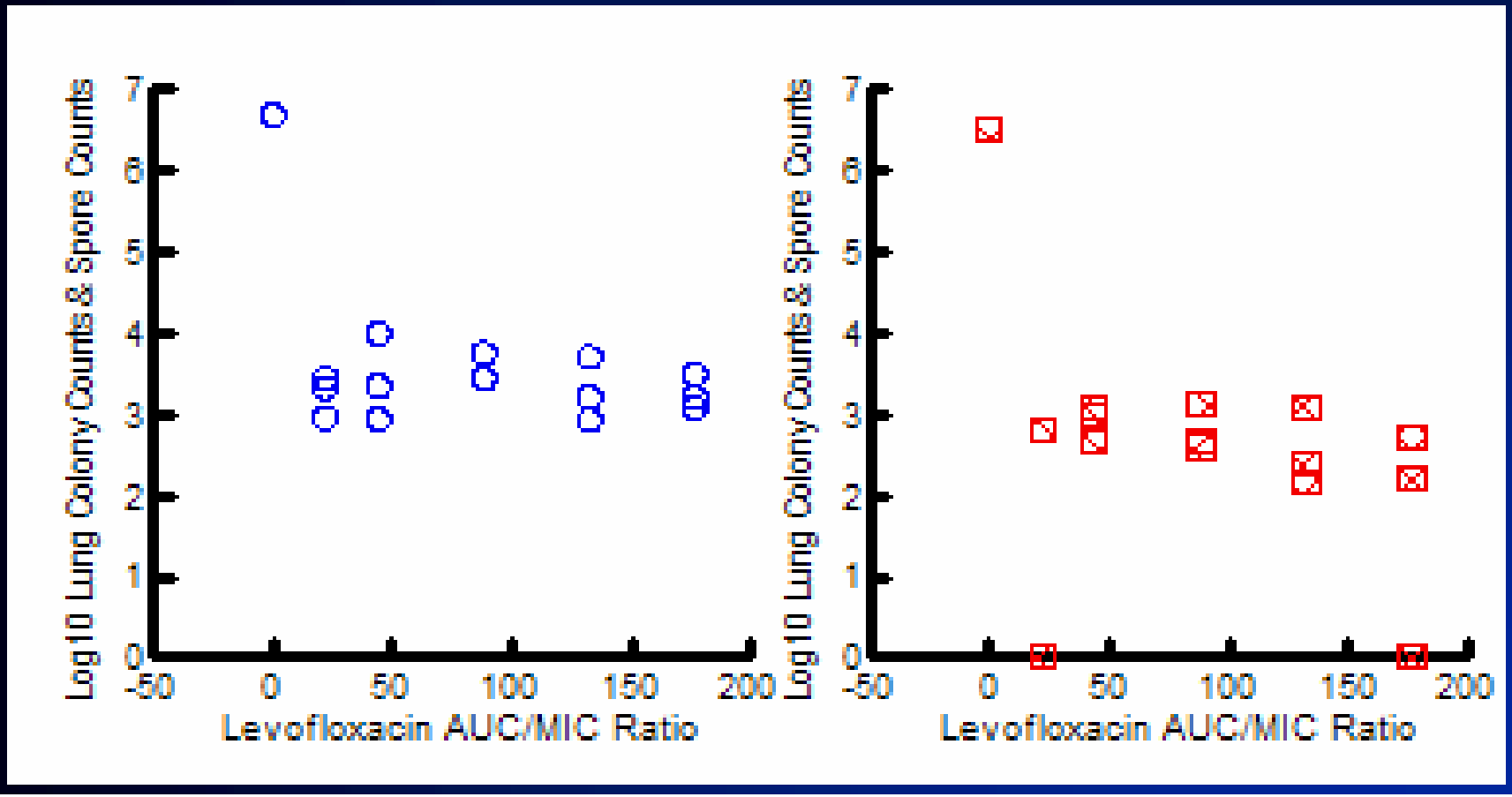


When Q24h dosing is removed, there is no difference by Schedule

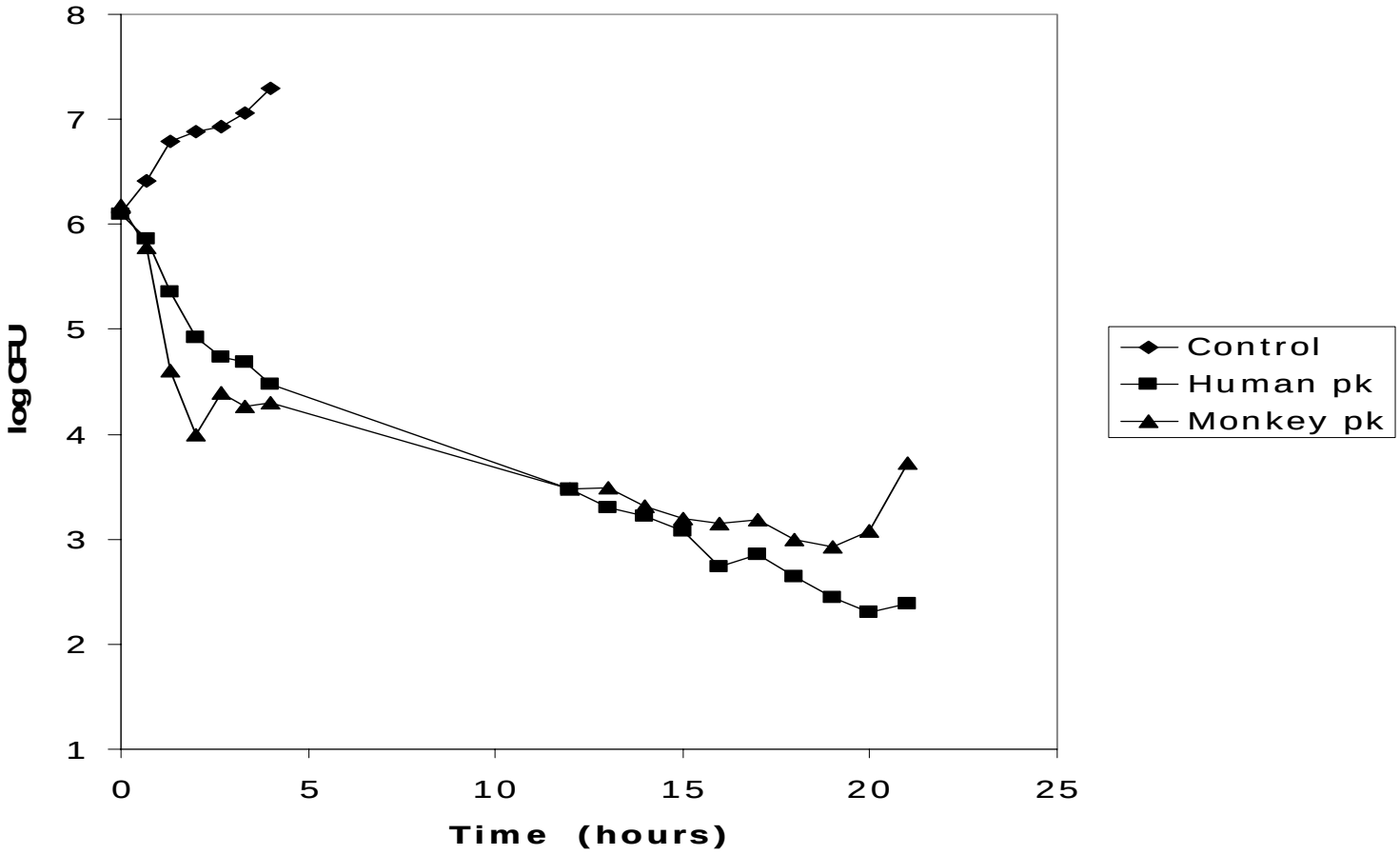
Now AUC/MIC is highly significant alone (p = 0.00012)

Total Counts

Spore Counts (heat shock)



Persistent Effect: Levo vs. B. anthracis



Preliminary Conclusions

- The mouse system showed that the adverse impact of Q24h dosing schedule predicted by the hollow fiber system did, indeed, occur
- Human PK and “Humanized” rhesus PK killed the organisms with NO emergence of resistance for levofloxacin
- When ciprofloxacin was administered as twice the exposure once daily (same 24 hour exposure), there was immediate (24 hour) emergence of resistance and regimen failure
- The ciprofloxacin failure with resistance is likely due to pump overexpression

Preliminary Conclusions

- The mouse model demonstrated good effect with relatively low AUC/MIC ratios on Q12h and Q6h dosing (likely due to an intact immune system)
- The levofloxacin failures with resistance were probably due to target site mutation (not proven)
- We are currently investigating the impact of sporulation

Preliminary Conclusions

- The rhesus challenge with Levo/Cipro Rx is taking place currently
- Good protection is being seen with “Humanized” levofloxacin dosing
- The hollow fiber model predicts both success (rhesus) and failure (mouse Q24h dosing)

Lab Animal Usage

Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Funding Acknowledgement

The research described herein was sponsored by the (ie. Medical Biological Defense Research Program, U.S. Army Medical Research and Materiel Command)] [Project/Grant/Agreement/Contract No. X].

Acknowledgements

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